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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of	:	Richard H. Tullis			
Appln. Serial No.	:	08/078,768	**Group No.**	:	1633
Filing Date	:	June 16, 1993	**Examiner**	:	J. Martinell

For : OLIGONUCLEOTIDE THERAPEUTIC AGENT AND METHODS OF MAKING SAME

DATE OF DEPOSIT: June 17, 2002
I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL, POSTAGE PREPAID ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE COMMISSIONER FOR PATENTS AND TRADEMARKS, WASHINGTON, DC 20231.

JOHN W. CALDWELL
REGISTRATION NO.: 28,937

DECLARATION OF DR. STANLEY T. CROOKE PURSUANT TO 37 CFR § 1.132

I, Dr. Stanley T. Crooke, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The Exhibit attached hereto is incorporated herein by reference.

2. I am Founder, Chairman and Chief Executive Officer of Isis Pharmaceuticals. I am currently a member of the Board of Directors of Antisense Therapeutics, Limited, Toorak, Victoria, Australia; Applied Molecular Evolutions, Inc., San Diego, California; EPIX Medical, Inc., Cambridge, Massachusetts; Idun Pharmaceuticals, Inc., La Jolla, California; and Axon Instruments, Inc., Union City, California. I also am a member of the IBC Advisory Council, Current Drugs Advisory Board, the Editorial Advisory Board of Journal of Drug

Targeting and Antisense Research and Development, and the Editorial Board of Gene Therapy and Molecular Biology. I also am Editor-in-Chief of Current Opinion in Anticancer Drugs and Section Editor for Biologicals and Immunologicals for Expert Opinion on Investigational Drugs. I have been appointed by the American Association for Cancer Research to serve as a member of the California State Legislative Committee.

I am an adjunct professor at University of California, San Diego and San Diego State University. I have authored over 425 publications and have edited 19 books.

Prior to founding Isis Pharmaceuticals, I was President of Research and Development for SmithKline Beckman Corporation (SKB). Prior to joining SKB, I helped establish the anticancer drug discovery and development program at Bristol Myers.

A copy of my *curriculum vitae* is attached as Exhibit 1.

3. As early as 1970, I worked with oligonucleotides and their uptake by cells. As early as 1971, I co-authored scientific journal articles regarding the same. In 1972, I published my doctoral thesis entitled "Preliminary studies on genetic engineering: The uptake of oligonucleotides and RNA by Novikoff hepatoma ascites cells." I was familiar at that time with phosphorothioate oligonucleotides. Indeed, in my doctoral thesis I referred to the work of DeClercq as disclosing, *inter alia*, increased stability of polynucleotides to RNAse rendered by "methods such as thiophosphate substitution." It was routine for me to refer to the works cited in a scientific article and to perform literature searches to provide the background for my research.

A routine literature search by one of ordinary skill in the art would have yielded a number of stabilized oligonucleotides, including phosphorothioates, alkylphosphonates, and phosphotriesters, available to those skilled in the art as of October, 1981.

4. I have read and am familiar with the contents of the above-referenced patent application. I have read and agree with the August 19, 1994 and April 14, 1995 declarations of Dr. Jerry L. Ruth and Dr. Dennis H. Schwartz. I further understand that the nature of the rejection at issue in the pending application is that the Examiner believes that the pending claims should be restricted to phosphotriester-modified nucleic acids which are included in the specification as a preferred embodiment as representing a class of stabilized oligonucleotides for *in vivo* applications. The Examiner alleges that the pending claims, calling for stabilized oligonucleotides, are overbroad.

The purpose of this declaration is to address this issue. In particular, I will explain that: (1) as of the priority date, one of ordinary skill in the art was aware of the existence of stabilized forms of oligonucleotides in addition to phosphotriesters and would have been guided by the disclosure of the application to the other stabilized oligonucleotides that are suitable, along with phosphotriester oligonucleotides, for *in vivo* use according to the claimed invention; (2) nothing more than routine experimentation is involved in determining which forms of stabilized oligonucleotides will work in the invention; (3) the stabilized oligonucleotides known in the art as of October 23, 1981 are taken up by cells, are sufficiently stable to exert biological activity, and specifically hybridize to their target mRNAs ; and (4) *in vitro* models correlate to *in vivo* biological activity of the stabilized oligonucleotides, as substantiated by pre- and post-filing references.

5. The application provides adequate guidance to one of ordinary skill in the art of the stabilized oligonucleotides known in the art and available prior to the October 23, 1981 filing date of the parent application.

a. Issue: The selection of the form of stabilization of the oligonucleotides for use in the invention is an inventive principle thereof and is not recited in the application.

The Facts: The point of novelty of the invention is not the form of stabilized oligonucleotides used therein. Indeed, various forms of stabilized oligonucleotides were known in the art in 1981, as discussed below. An inventive principle of Applicant's discovery is that protein expression may be specifically inhibited by targeting the coding region of a mRNA with an oligonucleotide of greater than about 14 nucleotides that is substantially complementary to the mRNA.

b. Issue: The application fails to guide those of skill in the art as to which oligonucleotides to use, as a literature search is not expected of one of skill in the art. The "scant statements" in the application are insufficient guidance as to which oligonucleotides to use in the invention.

The Facts: Stabilized oligonucleotides were known in the art in October 1981, and the application provides guidance as to which oligonucleotides to use. The language of the application made evident to one of ordinary skill in the art in 1981 that phosphotriester oligonucleotides were only a representative example of other forms of stabilized

oligonucleotides, all of which would have been equally suitable for use in the methods of the invention. To identify those other stabilized oligonucleotides, one of ordinary skill in the art need only begin with the application itself as the phosphotriester oligonucleotides set forth in the application as an example of stabilized oligonucleotides were described in the literature cited in the application. Specifically, the application cites Miller *et al.* (Biochemistry, 16:1988 (1977) ("Miller 1977")) for describing the stabilized, nuclease resistant phosphotriester form of oligonucleotides used in the examples. Thus, I am in agreement with the earlier-filed declarations of Dr. Ruth and Dr. Schwartz that the phosphotriester reference in the original application would have "lead one of skill directly to Dr. Paul Miller's work" and that "anyone familiar with Dr. Miller's work would have known of analogous work by Dr. Fritz Eckstein using thio-substituted nucleic acid." This is true because Miller et al. (Biochemistry, 13(24): 4887-4906 (1974)), referenced in the Miller 1977 article named in the application, specifically cites to the work of Eckstein.

It is my experience as a researcher that when reviewing a scientific article, one routinely refers to the works cited therein to provide the background against which the research is presented. Thus, the study of one scientific article routinely leads me to the review of several other such scientific articles.

Moreover, a routine literature search by one of ordinary skill in the art would have yielded a number of stabilized oligonucleotides available to those of ordinary skill in the art in October of 1981 and suitable for use in the invention. For example, U.S. Patent No. 3,687,808 to Merigan et al. describes stabilized phosphorothioate oligonucleotides available as early as 1972. In addition, Matzura and Eckstein (Eur. J. Biochem., 3: 448-452 (1968)) describe the nuclease resistance of phosphorothioate oligonucleotides. Agarwal and Riftina (Nuc. Acids Res., 6:9, 3009-3024 (1979)) describe the synthesis of oligonucleotides containing methyl and phenylphosphonate linkages. DeClercq et al. (Virology, 42:421-428 (1970)) describe the resistance of thiophosphate-substituted oligonucleotides to degradative enzymes. Befort et al. (Chem.-Biol. Interactions, 9:181-185 (1974) ("Befort")) report that ribonucleic acids stabilized by methylation are taken up by cells and exhibit anti-viral activity. Miller et al. (Biochem. 20(7): 1874-1880 (1981) ("Miller 1981")) report a stabilized alkyl phosphonate DNA analog having activity *in vitro*. Holý ("Synthesis and Biological Activity of Some Analogues of Nucleic Acids Components," in Phosphorus Chemistry Directed Towards Biology, W.J. Stec, Ed., Pergamon Press, 53-64, 1980) describes modified nucleotide analogs having hydroxyl-containing aliphatic chains that are stable *in vivo* and

display inhibitory and substrate activities. Harvey et al. (Biochem. 12(2):208-214 (1973) ("Harvey")) describe 5'-terminal alkyl phosphorothioate groups as protecting groups in oligonucleotide synthesis . Malkievicz et al. (Czech. Chem. Commun., 38:2953-2961 (1973) ("Malkievicz")) demonstrate the use of alkyl thioyl moieties as blocking groups in oligonucleotide synthesis.

I further agree with both Dr. Ruth and Dr. Schwartz that,

> The above references are representative of a significant body of work . . . . For the Examiner to maintain that those of skill would not have known of the above references or not have been able to find the above references is contrary to the way scientists work . . . .

April 14, 1995 declarations of Dr. Ruth and Dr. Schwartz at 4. Summerton et al. (J. Theor. Biol. 78:77-99 (1979) ("Summerton")), reviewing the modified oligonucleotides available in 1979, confirms that a person of ordinary skill in the art could have located the references describing stabilized oligonucleotides with only reasonable diligence as much as two years prior to Applicant's priority date.

Additionally, the rate of developments in the "field of chemical synthesis" of oligonucleotides in the early 1980s is irrelevant to the present analysis. That improvements to methods of chemically synthesizing oligonucleotides were being made in the early 1980s has no bearing on the patentability of the methods of selectively inhibiting the expression of a target protein in a cell without inhibiting the expression of other proteins.

Moreover, the rate of developments in the field of stabilization of oligonucleotides in the early 1980s was not so rapid, as evidenced by the broad timeframe over which the aforementioned publications became available and as further reflected by references published shortly after the 1981 priority date, that the level of skill in the art changed dramatically over the course of only a few months. For example, Miller et al. (Nucleic Acids: The Vectors of Life, 521-535 (1983) ("Miller 1983")), which was submitted for publication prior to the effective filing date, describe alkyl phosphotriester and methylphosphonate oligonucleotides as nuclease resistant analogs that are taken up by cells in culture. Likewise, Vosberg et al. (J. Biol. Chem., 257(11): 6595-6599 (1982) ("Vosberg")) and Connolly et al. (Biochem., 23(15):3443-3453 (1984) ("Connolly")) describe the nuclease resistance and resulting increased oligonucleotide stability through the use of phosphorothioate linkages.

Thus, I can state unequivocally that it is clear that, as of the October 23, 1981 priority date of the present application, those of ordinary skill in the art would have understood Applicant's reference to stabilized oligonucleotides to have included more than just the phosphotriester compounds that were specifically identified and that the application provides adequate guidance as to which stabilized oligonucleotides to use in the invention given the level of skill in the art.

6. The application provides adequate guidance to one of ordinary skill in the art as to how to use other stabilized oligonucleotides in the methods of the invention.

a. Issue: Undue experimentation would be required to practice the invention.

The Facts: Nothing more than routine experimentation was necessary to use the stabilized oligonucleotides known in the art in October 1981 in the invention. The methods for using different stabilized oligonucleotides according to the invention are essentially identical to the methods for using phosphotriester oligonucleotides set forth in the application. The stabilized oligonucleotide is simply administered, and the expression of the target protein is monitored. This experimentation was routine for one of ordinary skill in the art in 1981.

b. Issue: The application provides no guidance regarding the internalization of the stabilized oligonucleotides *in vivo*. Gura and Rojanasakul support the contention that not all stabilized oligonucleotides are internalized by cells. The post-priority date references cited by the applicant are not available to the applicant as rebuttal evidence.

The Facts: The specification provides adequate guidance regarding internalization of stabilized oligonucleotides by cells. In regard to cellular uptake of the stabilized oligonucleotides, I am in agreement with the August 19, 1994 declarations of Dr. Ruth and Dr. Schwartz. It was known in the art in 1981 that short oligonucleotides are internalized by cells in the absence of special culture conditions or methods to stimulate uptake thereof. This fact is evidenced by several pre-priority date references, including Befort, Miller 1981, and Summerton, each of which describes the uptake of modified oligonucleotides by cells. For example, the Befort article reports that "[a]ll RNAs, modified or not, enter the cells . . . ." Befort at 181. The Miller 1981 abstract states that "[t]ritium-labeled oligodeoxyribonucleoside methylphosphonates . . . are taken up intact by mammalian

cells in culture." Likewise, Summerton describes the uptake of DNA and RNA by prokaryotic and eukaryotic systems.

Moreover, I note that Gura is not a peer-reviewed article by a practicing scientist. Rather the article was written by a ***reporter* for the *Chicago Tribune***. Gura at 577. Gura merely represents a biased and untrained assessment of antisense technology by an individual having no skill in the art who solicited the ***opinions of admitted skeptics*** of the technology, including Cy Stein, Arthur Krieg, and others. Gura at 575. The news article contains no counterbalancing opinions by those who have been successful in oligonucleotide technology. The lengthy prosecution of the present application has allowed us to witness the successes achieved in the field of antisense technology, ratifying the views of proponents of antisense at the time of the invention and silencing, and indeed converting, many critics to what is clearly the correct view. ***Scientifically grounded, peer-reviewed journal articles*** support the fact that stabilized oligonucleotides are internalized by cells. These journal articles were authored by individuals of ordinary skill practicing nothing more than what is taught by the present application. In sum, Tullis was right then – true scientists knew it – and is right today.

The Rojanasakul article cited by the Examiner reports that, "[w]hen added directly to cells in culture, only 1-2% of the added [oligonucleotides] become cell-associated." Rojanasakul at 118, 120. Rojanasakul also states that there are "examples of successful in vivo treatment in the absence of specialized delivery systems." *Id.* at 118. Additionally, Rojanasakul reports that phosphorothioate oligonucleotides readily compete with unmodified oligonucleotides for cellular uptake and that methylphosphonates also enter cells. *Id.* at 120. Thus, Rojanasakul supports cellular uptake of stabilized oligonucleotides.

Additionally, Phillips et al. (Kidney International, 46: 1554-1556 (1994)) report the *in vivo* effects of phosphorothioated oligodeoxynucleotides simply injected into the brains of rats and concluded that "[o]ur present results do show that sufficient [oligodeoxynucleotide] uptake occurs in vivo to provide inhibition of blood pressure which appears to be related to the inhibition of angiotensin gene or AT_1 receptor gene expression." Phillips at 1556. Additionally, Hijiya et al. (Proc. Natl. Acad. Sci. 91: 4499-4503 (1994)) report that phosphorothioate-modified antisense oligodeoxynucleotides targeted to the MYB protooncogene controlled the growth of a human leukemia in a SCID mouse model. Thus, Phillips and Hijiya reinforce the operability of the claimed methods. Mercola et al. (Cancer Gene Therapy, 2(1): 47-59 (1995)) lends further support to the *in vivo* operability of stabilized oligonucleotides. Mercola describes several studies in which phosphorothioate

oligonucleotides administered systemically downregulate the *in vivo* expression of their targets. *See, e.g.,* Mercola at 54-55. Additionally, Putnam (Am. J. Health-Syst. Pharm. 53: 151-160 (1996)) describes several *in vivo* studies demonstrating cellular uptake of modified oligonucleotides. *See, e.g.,* Putnam at 154 ("When day-old Pekin ducklings were infected with duck hepatitis B virus and then, two weeks later, given daily intravenous injections of an antisense oligonucleotide for 10 days, there was reproducible dose-dependent inhibition of viral replication."); 156 ("In vivo studies in rats have also yielded promising results. Antisense oligonucleotides recognizing *c-myc*, cdk 2, and cdc 2 inhibited the proliferation of smooth muscle cells after carotid artery angioplasty.") (citations omitted). Thus, these post-priority date references confirm what was already known in October 1981 about the cellular uptake of stabilized oligonucleotides *in vivo*.

c. Issue: The application provides no guidance regarding the *in vivo* stability of modified oligonucleotides. The Examiner points to Gura and Rojanasakul to support the assertion that modified oligonucleotides are not stable *in vivo*.

The Facts: The Examiner relies on Gura to support his contention that modified oligonucleotides are not stable *in vivo*. Gura, however, poses the incorrect question. The appropriate question that should have been posed in that news article is not "do antisense compounds 'work the way researchers once thought they did'?" but rather "what are the pharmacokinetic data regarding the stability of modified oligonucleotides in cells and in an integrated system, such as an animal?" As presented herein, ***scientifically grounded, peer-reviewed journal articles*** support the fact that modified oligonucleotides are stable in cells. These journal articles were authored by individuals of ordinary skill practicing nothing more than what is taught by the present application.

The application provides adequate guidance regarding the stability of stabilized oligonucleotides. I am also in agreement with the April 14, 1995 declarations of Dr. Ruth and Dr. Schwartz stating that it was known in the art in October 1981 that modified oligonucleotides are stable in cells. For example, Befort reports the inhibition of Sindbis virus by chemically modified RNAs in chicken embryo fibroblasts.

Stability of modified oligonucleotides has been repeatedly confirmed by post-priority date references. Even Gura supports the stability of modified oligonucleotides *in vivo* by describing the many effects resulting from administration thereof in cell culture and *in vivo*. *See, e.g.,* Gura at 575 ("Researchers also made [the oligonucleotides] more resistant to the

many enzymes that break down nucleic acids by replacing a critical oxygen atom in each nucleotide building block with a sulfur atom."); 577 (describing the *in vivo* effects of phosphorothioate oligonucleotides). Likewise, Rojanasakul supports the *in vivo* stability of modified oligonucleotides. *See, e.g.,* Rojanasakul abstract (explaining that protected or modified oligonucleotides are stabilized against degradation in a biological environment); 119 (describing chemically modified oligonucleotides, including phosphorothioates, phosphorodithioates, and methylphosphonates, as preferred alternatives to naturally occurring phosphodiester oligonucleotides in view of *in vivo* stability). Phillips et al. (Kidney International 46: 1554-1556 (1994)) report that injections of phosphorothioated antisense oligodeoxynucleotides directed against angiotensin II type 1 mRNA into hypertensive rats produced a long lasting decrease in blood pressure. Additionally, Hijiya et al. (Proc. Natl. Acad. Sci. 91:4499-4503 (1994)) demonstrate that subcutaneous administration of phosphorothioated oligonucleotides targeting the MYB protooncogene suppressed MYB gene expression in a SCID mouse model. Mercola and Cohen (Cancer Gene Therapy 2(1): 47-59 (1995)) describe studies in which systemic delivery of modified oligonucleotides yields significant downregulation of expression of the targeted gene *in vivo*. Similarly, Putnam (Am. J. Health-Syst. Pharm. 53:151-160 (1996)) summarizes several studies in which modified oligonucleotides exhibit inhibitory effects *in vivo*. Thus, these post-priority date references confirm that which was already known to the ordinarily skilled artisan in 1981 regarding the stability of the modified oligonucleotides *in vivo*.

d. Issue: The application provides no guidance regarding the specificity of hybridization of the modified oligonucleotides *in vivo*. Gura and Rojanasakul support the contention that not all stabilized oligonucleotides bind.

The Facts: The Examiner relies on Gura to support his contention that stabilized oligonucleotides do not hybridize. Gura, however, poses the incorrect question. The appropriate question to be answered is "what are the specificity indices of stabilized oligonucleotides in cells and in an integrated system, such as an animal?" In fact, as presented herein, ***scientifically grounded, peer-reviewed journal articles*** support the fact that stabilized oligonucleotides hybridize specifically. These journal articles were authored by individuals of ordinary skill practicing nothing more than what is taught by the present application.

The application provides adequate guidance regarding the hybridization specificity of stabilized oligonucleotides. Given the representative example of a stabilized oligonucleotide in the application, only routine experimentation by one of ordinary skill in the art would be required to determine which other forms of stabilized oligonucleotides demonstrate specific hybridization *in vivo* using the application as a guide. It was known in the art in 1981 that stabilized oligonucleotides, including RNA and DNA analogs, bind specifically to their targets in cell culture. For example, Miller 1981 teaches that phosphonate analogs specifically bind intracellularly to initiation sites and tRNA binding sites of mRNA. Additionally, Befort uses artificially methylated nucleic acids to inhibit viral replication in cell culture. The Summerton reference also states that analogs and derivatives of nucleic acids function as a result of specific base pairing:

> [T]here are a growing number of reports on antiviral and/or anticancer activity of homopolyribonucleotides, analogs, and derivatives thereof, and a synthetic oligodeoxy-ribonucleotide. The general rationale for this work is that the introduction of such polymers into virally infected cells may lead to pairing between the introduced polymer and a specific viral structure of nucleotide sequence. Presumably such pairing would inhibit some critical function in the virus life cycle.

Summerton at 89.

The specificity of hybridization of stabilized oligonucleotides, including both RNA and DNA analogs, has been reinforced by a number of post-priority date publications. For example, the modified oligonucleotides of Phillips and Hijiya caused downregulation of expression of their target proteins. Mercola et al. (Cancer Gene Therapy, 2(1): 47-59 (1995)) and Putnam (Am. J. Health-Syst. Pharm., 53: 151-160 (1996)) lend further support to the *in vivo* operability of stabilized oligonucleotides. Mercola describes several studies in which phosphorothioate oligo*deoxy*nucleotides downregulate the *in vivo* expression of their targets. *See, e.g.,* Mercola at 54-55. Likewise, Putnam states that "[m]odification of the phosphodiester linkages in oligonucleotides can lend the sequences enzymatic stability without affecting their binding capacities." Putnam, abstract. The modifications referred to in Putnam include phosphorothioates, methylphosphonates, methylphosphotriesters, ethylphosphotriesters, and alkylphosphoramides. Putnam at 157, Figure 3. Thus, these post-priority date references confirm what was known to one of ordinary skill in the art in 1981.

In summary, (1) as of the priority date, one of ordinary skill in the art was aware of the existence of stabilized forms of oligonucleotides other than phosphotriesters and was guided by the disclosure of the application to the other stabilized oligonucleotides that are equally suitable as phosphotriester oligonucleotides for use according to the claimed invention; (2) nothing more than routine experimentation is involved in determining which forms of stabilized oligonucleotides will work in the invention; (3) the stabilized oligonucleotides known in the art at the effective filing date are taken up by cells, are sufficiently stable to exert biological activity, and specifically hybridize to their target mRNAs; and (4) the *in vitro* models correlate to *in vivo* biological activity of the stabilized oligonucleotides, as substantiated by pre- and post-filing date references.

I further declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 6/14/02

By: ______________________

Dr. Stanley T. Crooke